

In the Specification:

Please replace the paragraph beginning at page 3, line 16, with the following:

--FIG. 2 is a graph illustrating the adhesion of MDA-MB-435 breast carcinoma cells to recombinant thrombospondin-1 (TSP1) fragments and TSP1 peptides. Adhesion to synthetic TSP1 peptides adsorbed at 10 μ M (Peptide 246, KRFKQDGGWSHWSPWSS (SEQ ID NO:1); 500, NGVQYRNC (SEQ ID NO:2); Mal II, SPWSSCSVTCGDGVITRIR (SEQ ID NO:3); 4N1K, KRFYVVMWKK (SEQ ID NO:4); HepI, ELTGAARKGSGRRLVKGPD (SEQ ID NO:5)), TSP1 (0.11 μ M), recombinant 18 kDa heparin-binding domain (HBD, 2.7 μ M), or GST-fusion proteins expressing the TSP1 procollagen domain, type 1, type 2, type 3 repeats, or GST alone (2 μ M) was measured in the absence (solid bars) or presence of 20 μ g/ml of the β 1 integrin-activating antibody TS2/16 (striped bars). Results (mean \pm SD) are presented for a representative experiment performed in triplicate.--

Please replace the paragraph beginning at page 3, line 26, with the following:

--FIGS. 3A and 3B are graphs illustrating the adhesion of MDA-MB-435 breast carcinoma cells to TSP1 peptides and laminin-1 peptide GD6. Panel A: MDA-MB-435 breast carcinoma cell attachment (closed symbols) and spreading (open symbols) was determined on polystyrene substrates coated with the indicated concentrations of TSP1 peptide 678 (FQGV LQNVR FVF (SEQ ID NO:6), circles), TSP1 peptide 701 (TPGQVRTLWHDP (SEQ ID NO:7), squares), or the murine laminin-1 peptide GD6 (KQNCLSSRASFRGCVRNLRLSR (SEQ ID NO:8), triangles). Results are presented as mean \pm SD, n = 3. Panel B: Spreading of MDA-MB-435 or MDA-MB-231 cells on substrates coated with 3.3 μ M TSP1 peptide 678, 1.1 μ M laminin-1 peptide GD6, or 50 μ g/ml TSP1 was determined using untreated cells (solid bars), or cells treated

with 5 μ g/ml of the β 1 activating antibody TS2/16 (gray bars), or 3 nM IGF1 (striped bars, MDA-MB-435 cells only), mean \pm SD, n = 3.--

Please replace the paragraph beginning at page 4, line 28, with the following:

--FIG. 6 is a histogram showing the determination of the minimal active TSP1 sequence to promote breast carcinoma cell adhesion. MDA-MB-435 cell adhesion was determined to polystyrene coated with 10 μ M of the indicated TSP1 peptides (SEQ ID NOS:6, 31, 41, 40, 30, 32, 39 and 56, respectively) or with bovine serum albumin (BSA). Cell attachment is presented as the mean \pm SD for triplicate determinations.--

Please replace the paragraph beginning at page 5, line 7, with the following:

--FIG. 8 is a histogram showing the effect of systematic substitution of Ala residues on adhesive activities of the TSP1 sequence 190-201 (SEQ ID NOS:6, 25, 26, 27, 29, 10, 42, 11, 44, 43 and 28, respectively) for breast carcinoma cells. Cell attachment was determined to substrates coated with each peptide at 10 μ M and is presented as mean \pm SD, n = 3. Residues substituted in the native TSP1 sequence are indicated with an asterisk.--

Please replace the paragraph beginning at page 5, line 29, with the following:

--FIGS. 11A and 11B display adhesion of endothelial cells on an α 3 β 1 integrin-binding peptide from TSP1. Panel A: TSP1 peptide 678 (FQGV LQNVR FVF; SEQ ID NO:6) or analogs of this peptide with the indicated Ala substitutions (★) were adsorbed on bacteriological polystyrene substrates at 10 μ M in PBS. Direct adhesion of BAE cells to the adsorbed peptides or uncoated substrate (control) are presented as mean \pm SD, n = 3. Panel B: Loss of cell-cell contact stimulates endothelial cell spreading on TSP1. Two flasks of BAE cells were grown to confluence. One flask was harvested and

replated in fresh medium at 25% confluency. Fresh medium was added at the same time to the second flask. After 16 h, cells from both flasks were dissociated using EDTA and adhesion was measured on substrates coated with 40 $\mu\text{g/ml}$ TSP1, 10 $\mu\text{g/ml}$ vitronectin, 20 $\mu\text{g/ml}$ plasma fibronectin, or 5 $\mu\text{g/ml}$ type I collagen. The percent spread cells after 60 min is presented as mean \pm SD, $n = 3$ for a representative experiment.--

Please replace the paragraph beginning at page 6, line 23, with the following:

--FIGS. 14A, 14B and 14C display $\alpha 3 \beta 1$ and $\alpha v \beta 3$ integrin-mediated spreading of endothelial cells on thrombospondin-1. Panel A: BAE cell adhesion to TSP1 (solid bars), vitronectin (striped bars), or plasma fibronectin (open bars) was measured in the presence of 30 μM TSP1 peptide 678, 1 μM of the $\alpha v \beta 3$ integrin antagonist SB223245, 300 μM of the integrin antagonist peptide GRGDSP (SEQ ID NO:9), or the indicated combinations. Results are expressed as percent of the response for untreated cells, mean \pm S.D., $n = 3$. Panel B: HUVEC spreading on substrates coated with TSP1 (solid bars) or vitronectin (striped bars) was determined in the presence of 20 μM peptide 678, 1 μM $\alpha \text{IIb} \beta 3$ antagonist SB208651, 1 μM $\alpha v \beta 3$ antagonist SB223245, or 20 μM peptide 678 plus 1 μM SB223245. Spreading is presented as a percent of the respective controls without inhibitors (31 cells/mm² for TSP1 and 10 cells/mm² for vitronectin). Panel C: Inhibition of HDME cell spreading on TSP1 (solid bars) or type I collagen (striped bars) was determined in the presence of the indicated function blocking antibodies at 5 $\mu\text{g/ml}$: anti-CD36 (OKM5), anti-integrin $\beta 1$ (mAb13), anti-integrin $\alpha 3$ (P1B5), and anti-integrin $\alpha 4$ (P4C2).--

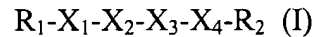
Please replace the paragraph beginning at page 8, line 4, with the following:

--FIGS. 19A, 19B, 19C, 19D and 19E display the modulation of endothelial cell proliferation by an $\alpha 3 \beta 1$ integrin binding peptide from TSP1. FIG. 19A: Proliferation of BAE cells was assayed in the presence of the indicated concentrations of

TSP1 peptide 678 (FQGV LQNVRFVF (SEQ ID NO:6),) or the control peptides 686 (FQGV LQAVRFVF (SEQ ID NO:10), \blacktriangle), and 690 (FQGV LQNVAFVF (SEQ ID NO:11), O). Briefly, 100 μl of a 5×10^4 cell/ml suspension of BAE cells were seeded in triplicate into 96 well tissue culture plate in DMEM medium containing 1% FCS, 10 ng/ml of FGF2 and peptides at 1-40 μM concentrations. Cells were incubated for 72 h, and proliferation was measured using the Celltiter tetrazolium assay (Promega). FIG. 19B: HUVE cell proliferation was measured at 72 h for cells plated on wells coated with the indicated concentrations of TSP1 (solid bars) or 1 $\mu\text{g/ml}$ of antibody P1B5 (anti- $\alpha 3 \beta 1$ integrin) (striped bar) or P1D6 (anti- $\alpha 5 \beta 1$ integrin) in medium 199 containing 5% FCS (striped bar). FIG. 19C: $\alpha 3 \beta 1$ integrin mediates the proliferative response to immobilized TSP1. HUVE cells were plated in medium 199 containing 20% FCS on wells coated using 5 $\mu\text{g/ml}$ TSP1, 5 $\mu\text{g/ml}$ vitronectin, or BSA (control) alone or in the presence of 5 $\mu\text{g/ml}$ of the $\alpha 3 \beta 1$ blocking antibody P1B5 or 20 μM of TSP1 peptide 678. Proliferation was determined at 72 h, and is presented as a percent of the control, mean \pm S.D., $n = 3$ for experimental points and $n = 6$ for control. FIG. 19D: HUVE cell proliferation was determined in the presence of the indicated concentrations of TSP1 peptide 678 immobilized on the substrate (solid bars) or added in solution (striped bars). Conditions that significantly differed from their respective controls based on a 2-tailed t test with $p < 0.05$ are marked with an “*”. FIG. 19E: HDME cell proliferation in MCDB growth medium with 5% FCS was determined in the presence of 10 ng/ml FGF2 and the indicated concentrations of TSP1 added in the medium (Δ) or immobilized on the substrate (\bullet) or in wells coated with the indicated concentrations of peptide 678 (\blacktriangle). Results are presented as mean \pm S.D. and are normalized to controls without TSP1 or peptide.--

Please replace the paragraph beginning at page 16, line 22, with the following:

--The present invention generally provides peptides, comprising the sequence



wherein X1 is selected from the group consisting of N, Q, D and S; X2 is selected from the group consisting of V, I and L; X3 is selected from the group consisting of R and K; and X4 is selected from the group consisting of V, I, L and F; R1 is a hydrogen or a peptide of 1 to 6 amino acids, an acyl or an aryl group; and R2 is a peptide of 1 to 3 amino acids, a hydroxide or an amide. In one embodiment of the invention, peptides having the sequence FQGV LQNVR FVF (SEQ ID NO:6) or FRGCVRNLRLSR (SEQ ID NO:12) are specifically excluded. In one embodiment, the peptides contain from 4 to 12 amino acids, i.e., has a length of 4 to 12 amino acid residues. In one embodiment, the peptides comprise additional residues, e.g., typically up to a length of 15, 20, 25, or 40 residues that includes the core sequence (I).--

Please replace the paragraph beginning at page 17, line 1, with the following:

--In one embodiment of the present invention, R₁ is a peptide comprising the sequence selected from the group consisting of FQGV LQ (SEQ ID NO:13), FAGV LQ (SEQ ID NO:14), FQGV AQ (SEQ ID NO:15), FQGV LA (SEQ ID NO:16), and FQGV LN (SEQ ID NO:17).--

Please replace the paragraph beginning at page 17, line 4, with the following:

--In one embodiment, the peptide of the present invention comprises at least one sequence selected from the group consisting of FQGV LQNLR FVF (SEQ ID NO:18), FQGV LQDVR FVF (SEQ ID NO:19), FQGV LQQVR FVF (SEQ ID NO:20), FQGV LQSVR FVF (SEQ ID NO:21), acQGV LQNVRF (SEQ ID NO:22),

FQGV LQNVKFVF (SEQ ID NO:23), FQGV LNNVRFVF (SEQ ID NO:24),
AQGV LQNVRFVF (SEQ ID NO:25), FAGV LQNVRFVF (SEQ ID NO:26),
FQGV AQNVRFVF (SEQ ID NO:27), FQGV LQNVRFVA (SEQ ID NO:28),
FQGV LANVRFVF (SEQ ID NO:29), FQGV LQNVRFV (SEQ ID NO:30),
QGV LQNVRFVF (SEQ ID NO:31), FQGV LQNVRF (SEQ ID NO:32), and
FQGV LQNVRFVF (SEQ ID NO:6).--

Please replace the paragraph beginning at page 25, line 31, with the following:

--The peptide GRGDSP (SEQ ID NO:9) was obtained from Gibco/BRL.
A non-peptide antagonist of αv integrins was provided by Dr. William H. Miller
(SmithKline Beecham Pharmaceuticals, King of Prussia, PA) (Keenan, 1997).--

Please replace the paragraph beginning at page 30, line 24, with the following:

--A multiple alignment search using MACAW software was used to
identify TSP1 sequences that might be related to the $\alpha 3 \beta 1$ integrin-binding murine
laminin-1 peptide KQNCLSSRASFRGCVRNLRLSR (GD6 peptide; SEQ ID NO:8)
derived from the A chain of murine laminin-1 (Gehlsen et al., 1992), which strongly
promoted MDA-MB-435 cell adhesion (**FIG. 3A**). This search identified four TSP1
sequences related to the laminin peptide (**Table 1**).--

Please replace the paragraph (**Table 1.**) beginning at page 31, line 1, with the following:

--Table 1. TSP1 sequences related to murine laminin-1 peptide GD6.

The amino acid sequences for human and murine TSP1 and laminin-1 peptide GD6 were compared by multiple alignment using MACAW. Alignment scores were determined by segment pair overlap or Gibbs sampler (*) methods.

Peptide origin	sequence	MP score vs. GD6	p value	SEQ ID NO:
laminin GD6	KQNCLSSRASFRGCVRNLRLSR	-	-	8
laminin p679	FRGCVRNLRLSR	-	-	12
TSP1(598-608)	NCLPCPPRFTG	42.0	5.9x10-8	33
TSP1(188-199)	DNFQGVQLQNVRF	39.0	5.9x10-7	34
TSP1(392-405)	NNRCEGSSVQTRTC	35.0	4.5x10-4	35
TSP1(1059-1077)	RNALWHTGNTPGQVRTLWH	43.3*	2.1x10-8	36

Please replace the paragraph (**Table 2.**) beginning at page 33, line 8, with the following:

--Table 2. Inhibition of MDA-MB-435 cell adhesion to immobilized peptide 678 by peptide analogs of TSP1 as well as direct adhesion of immobilized peptide analogs to MDA-MB-435 cells.

Mean doses to achieve 50% inhibition of control adhesion (IC₅₀) to polystyrene coated with 5 μ M peptide 678 were determined from at least three independent experiments, each performed in triplicate. Peptides were tested at up to 300 μ M or to the solubility limit for each peptide where lower limits for inhibitory activity are indicated.

#	Sequence (SEQ ID NO:)	MW	Source	Inhibition of peptide 678 (IC ₅₀)	Direct adhesion#
674	GEFYFDLRLKGDKY (37)	1751	type IV coll.		
675	KQNCLSSRASFRGCVRNLRLSR (8)	2552	laminin GD6		+++
678	FQGV LQNVR FVF (6)	1454	TSP1	3.5((15))	+++
679	FRGCVRNLRLSR (12)	1477	part of GD6	((700))	+++
681	ac-LQNVR F-am (38)	815	part of 678	500	-
682	FQGV LQNVR F (32)	1207		6	++
683	QGV LQNVR (39)	913		>300	-
685	QGV LQNVR FVF (31)	1307		24	++
684	LQNVR FVF (40)	1022		300	+/-
688	VLQNVR FVF (41)	1121		>100	+
689	FQGV LQNVR FV (30)	1307		+	+
686	FQGV LQAVR FVF (10)	1411		>300	++
687	FQGV LANVR FVF (29)	1397		3	++
690	FQGV LQNVAFVF (11)	1369		>300	-
691	FQGV LQNAR FVF (42)	1426		>300	++
692	FQGV LQNVRAVF (43)	1378		18	++
693	FQGV LQNVR FVA (28)	1378		27	++
694	FQGV LQNVHFVF (44)	1435		54	+/-
695	FQGV AQNVR FVF (27)	1412		5	++
696	FAGV LQNVR FVF (26)	1397		1.8((12))	++
697	AQGV LQNVR FVF (25)	1378		5	++
698	FQGV LNNVR FVF (24)	1440		3	++
701	TPGQVRTLWHDP (7)	1407	(part of C6)	>300	-
702	FQGV LQNVKFVF (23)	1426		6((25))	+++
703	FQGV LQNVQFVF (45)	1426		>100((300))	+/-
704	acQGV LQNVR F (22)	1060		15((~100))	++
705	FQGV LQSVR FVF (21)	1427		((15))	++
709	D reverse-678 (-)			((18))	
716**	carboxamidomethyl-thiopropionyl- FQGV LQNVR FVF (46)	1538		((100))	
717	FQGV LQQVR FVF (20)	1468		((30))	
718	FQGV LQDVR FVF (19)	1455		((12))	
719	FQGV LQNLRFVF(18)	1468		((16))	

* Inhibition constants (IC₅₀) were determined by microscopic adhesion assays except where indicated by (()) in which case the inhibition constants were determined by the hexosaminidase hexosaminidase method.

#Activity to promote MDA-MB-435 cell adhesion in a direct adhesion assay using peptides adsorbed on polystyrene.--

Please replace the paragraph (**Table 3.**) beginning at page 36, line 1, with the following:

--Table 3. Mapping of essential residues for inhibition of MDA-MB-435 cell adhesion to immobilized peptide 678.

Mean doses to achieve 50% inhibition of control adhesion to 5 μ M peptide 678 (IC₅₀) were determined from at least three independent experiments, each performed in triplicate. Residues substituted in the native TSP1 sequence are underlined. substituted in the native TSP1 sequence are underlined.

Peptide	Sequence	SEQ ID NO:	IC ₅₀ (μ M)
678	FQGV L Q N VR F V F (TSP1)	6	3.5
697	<u>A</u> QGV L Q N VR F V F	25	5
696	F <u>A</u> GV L Q N VR F V F	26	1.8
695	FQGV <u>A</u> Q N VR F V F	27	5
687	FQGV L <u>A</u> NVR F V F	29	3
686	FQGV L Q <u>A</u> VR F V F	10	>300
691	FQGV L Q N <u>A</u> R F V F	42	>300
690	FQGV L Q N V <u>A</u> F F V F	11	>300
702	FQGV L Q N V <u>K</u> F F V F	23	6
694	FQGV L Q N V <u>H</u> F F V F	44	54
703	FQGV L Q N V <u>Q</u> F F V F	45	>100
692	FQGV L Q N VR <u>A</u> V F	43	18
693	FQGV L Q N VR F V <u>A</u>	28	27

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Please replace the paragraph beginning at page 37, line 22, with the following:

--Based on examination of synthetic peptides and recombinant fragments representing approximately 90% of the TSP1 sequence, only the sequence

FQGV LQNVR FVF (SEQ ID NO:6) from the amino terminal domain exhibited activities that are expected for an $\alpha 3 \beta 1$ integrin binding sequence in TSP1. A recombinant fragment of TSP1 containing this sequence also promoted $\beta 1$ integrin-dependent adhesion. In solution, this peptide specifically inhibited adhesion to TSP1 but not to ligands recognized by other integrins. Adhesion to this peptide and to TSP1 was stimulated by IGF1 receptor ligands that stimulate integrin-dependent adhesion to intact TSP1, by Mn^{2+} , and by a $\beta 1$ integrin-activating antibody and partially inhibited by an $\alpha 3 \beta 1$ function blocking antibody. Based on systematic amino acid substitutions in the active sequence, NVR appears to be the essential core sequence in this TSP1 peptide for recognition by the $\alpha 3 \beta 1$ integrin.--

Please replace the paragraph beginning at page 37, line 33, with the following:

--Adhesive activities of the immobilized peptides imply that only Arg(198) may directly participate in this interaction, although the partial resistance to inhibition by an $\alpha 3 \beta 1$ integrin antibody and EDTA suggest that the peptides with Arg may also support adhesion independent of integrin binding. The context surrounding the Arg is important, however, because other peptides with similar sequences (such as peptide 701 with a QVRT (SEQ ID NO:47) sequence) had no activity, and Ala substitutions of the flanking residues in peptide 678 eliminated or markedly decreased its inhibitory activity in solution. The essential amino acid residues are completely conserved in human, murine, bovine, and Xenopus TSP1, although in chicken TSP1 a His replaces the Arg. A similar motif is found in murine and human TSP2, with a His residue replacing the Arg. As a free peptide the TSP1 sequence with a His substitution was much less active, so it is not clear whether the TSP2 sequence could be recognized by $\alpha 3 \beta 1$ integrin. Activity of the latter sequence may be increased in an environment that increases protonation of the imidazole in His.--

Please replace the paragraph beginning at page 38, line 12, with the following:

--A consensus $\alpha 3 \beta 1$ integrin recognition sequence in $\alpha 3 \beta 1$ ligands has not been reported. One hypothesis is that different ligands have unrelated binding sequences, which is supported by a recent mutagenesis study (Krukonis et al., 1998). However, other recent data has raised questions about whether all of the proteins reported to mediate $\alpha 3 \beta 1$ -dependent adhesion are true $\alpha 3 \beta 1$ ligands (Krukonis et al., 1998). LamA2 and LamA3 were verified to bind $\alpha 3 \beta 1$ integrin and have potential binding motifs based on the data, but human LamA1, which was found not to bind $\alpha 3 \beta 1$ with high avidity, has an Ala in the position occupied by the essential Arg in the TSP1 sequence. Substitution of Ala for the Arg in the TSP1 sequence abolished all activity of the synthetic TSP1 peptide. Although RGD was reported to be an $\alpha 3 \beta 1$ ligand, the RGD in entactin is not required for recognition. A binding site for the $\alpha 3 \beta 1$ integrin in entactin was mapped to the G2 domain (residues 301-647) (Gresham et al., 1996). Multiple alignment of this region of entactin against the TSP1 sequence and the murine laminin-1 peptide GD6 identified a related sequence FSGIDEHGHITI (SEQ ID NO:48), but this sequence lacks any of the essential residues in the TSP1 sequence. This domain of entactin also contains two NXR sequences: NNRH (SEQ ID NO:49) and NGRQ (SEQ ID NO:50). It remains to be determined whether either of these can function as an $\alpha 3 \beta 1$ integrin recognition sequence.--

Please replace the paragraph beginning at page 41, line 11, with the following:

--The increased spreading of BAE cells on TSP1 is mediated at least in part by $\alpha 3 \beta 1$ integrin, because a TSP1 peptide that binds to this integrin inhibited spreading on TSP1 by 55% but did not inhibit spreading on fibronectin or vitronectin substrates (**FIG. 14A**). The $\alpha v \beta 3$ integrin also plays some role in BAE cell spreading on TSP1, since the αv integrin antagonist SB223245 partially inhibited spreading on TSP1. The effect of these two inhibitors was additive, producing a 76% inhibition of spreading

when combined. Similar results were obtained using the $\alpha v \beta 3$ peptide antagonist GRGDSP (SEQ ID NO:9) alone and in combination with peptide 678. Approximately 20% of the spreading response on TSP1 was resistant to the GRGDSP (SEQ ID NO:9) peptide, but combining this peptide with the $\alpha 3 \beta 1$ binding peptide completely inhibited spreading on TSP1.--

Please replace the paragraph beginning at page 46, line 30, with the following:

--The $\beta 1$ integrin recognition sequence in TSP1 may also contribute to angiogenesis, because peptide 678 (FQGV LQNVR FVF; SEQ ID NO:6) inhibited angiogenesis in the CAM assay (**Table 5**). The results of dose dependent inhibition of angiogenic response for various peptides (including peptide 678) are presented in **Table 5**.--

Please replace the paragraph (**Table 5.**) beginning at page 47, line 1, with the following:

--Table 5. Angiogenetic response (% inhibition) of various peptides determined by chick chorioallantoic membrane (CAM) angiogenesis assay.

Vitrogen gels containing peptides at the indicated concentrations were placed on CAMs in triplicate. Angiogenesis was assessed by injecting FITC-dextran after 24 h and imaging the resulting vascular bed in the gels. Results are presented as percent inhibition relative to control gels without peptides, mean \pm SD.